

Sub d1  
22. A method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, the method comprising the steps of:

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- a. coating paramagnetic particles or beads with a first antibody or antibody fragment directed against the second antibody or antibody fragment;
  - b. incubating the second antibody or antibody fragment with the cell mixture to bind the antibody or antibody fragment with the target cell, wherein the second antibody or antibody fragment is directed against a membrane structure specifically expressed on the target cell and not on a non-target cell in the cell mixture;
  - c. washing the cell mixture to remove unbound second antibody or antibody fragment;
  - d. mixing the coated paramagnetic particles or beads with the washed cell mixture;
  - e. incubating the mixture under gentle rotation at about 4°C until target cell-bead rosettes are formed; and
  - f. visually detecting the target cell-bead rosettes after incubation.

Sub d3  
39. The method of claim 22, wherein the monoclonal antibody or antibody fragment is directed against fibronectin receptor,  $\beta$ -integrin, vitronectin receptor,  $\alpha\beta$ -integrin, P-selectin including GMP-140, CD44-variants, N-CAM including CD-56, E-cadherin, Le<sup>x</sup>, carcinoembryonic antigen or CEA, EGF receptor, c-erbB-2 including HER2, transferin receptor, TNF-receptor, molecular weight antigen, p95-100, sarcoma antigens including TP-1 and TP-3 epitope, Mv 200kD, Mv160kD, MOC-31 epitope including cluster 2 epithelial antigen, MUC-1 antigen including DF3-epitope and gp290kD, prostate molecular antigen, TAG 72, bladder carcinoma antigen, Mv 48kD colorectal carcinoma antigen, lung carcinoma antigen Mv 350-420kD, Mel-14 epitope,  $\beta_2$ -microglobulin, Apo-1 epitope, or pan-human cell antigen.

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46. A kit for performing the method of claim 22, the kit comprising:

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a. a specific monoclonal antibody or antibody fragment directed to an antigen on a target-cell, which monoclonal antibody or fragment is capable of coating a paramagnetic particle or bead without removing its antigen-binding ability;

b. a paramagnetic particle or bead; and

c. a second specific monoclonal antibody or antibody fragment directed against an antigen or a receptor within or on the target cell;

wherein said second antibody or antibody fragment is conjugated to a detectable label.

48. A method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, the method comprising the steps of:

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- a. coating paramagnetic particles or beads with a first antibody directed against a second antibody or antibody fragment;
- b. incubating the second antibody or antibody fragment with the cell mixture to bind the antibody or antibody fragment with the target cell, wherein the second antibody or antibody fragment is directed against a membrane structure specifically expressed on the target cell and not on a non-target cell in the cell mixture;
- c. washing the cell mixture to remove unbound second antibody or antibody fragment;
- b. mixing the coated paramagnetic particles with the washed cell mixture
- c. incubating the mixture under gentle rotation at about 4°C until target cell-bead rosettes are formed; and
- d. visually detecting the target cell-bead rosettes.

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78. A kit for performing the method of claim 111, the kit comprising:

a. a first monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell;

b. a second antibody directed against an Fc-portion of the first monoclonal antibody or fragment thereof;

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- c. a paramagnetic particle or bead; and
  - d. a labeled third specific monoclonal antibody directed against an antigen or a receptor within or on the target cell.

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G6

62. The method of claim 48, wherein when the density of target-cells is low, or when the ratio of target cell/total cells in the cell mixture is  $\leq 1\%$ , the method further comprises after incubating, applying a magnetic field to separate out the target cell-bead rosettes.

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87. A method for detecting tumor cells in a cell suspension of mixed cell population or in a cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, comprising:

- a) coating paramagnetic particles with a first antibody or fragment directed against a second a tumor-specific monoclonal antibody or fragment;
- b) incubating the second tumor specific antibody with the cell suspension to allow the second tumor specific antibody to bind the tumor cells;
- c) washing the cell suspension to remove unbound second antibody or antibody fragment;
- d) mixing the coated paramagnetic particles with the cell suspension;
- e) incubating the mixture at about  $4^{\circ}\text{C}$  under gentle rotation until tumor cell-bead rosettes are formed; and
- f) visually detecting the tumor cell-bead.

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92. A method of detecting metastatic cancer cells in a suspension of a mixed cell population or in a single cell suspension from a solid tissue when the metastatic cancer cells are present at less than 1% of the cell suspension, the method comprising the steps of:

- a) coating paramagnetic particles with a first antibody or fragment thereof directed against a second a cancer-specific monoclonal antibody or fragment;
- b) incubating the second tumor specific antibody with the cell suspension to allow the second tumor specific antibody to bind the tumor cells;
- c) washing the cell suspension to remove unbound second antibody or antibody fragment;

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- d) mixing the coated paramagnetic particles or beads with the cell suspension;
  - e) incubating the mixture under gentle rotation at about 4°C until tumor cell-bead rosettes are formed;
  - f) applying a magnetic field to separate out the tumor cell-bead rosettes; and
  - g) visually detecting the tumor cell-bead rosettes.
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Please add the following new claims.

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108. The method according to claim 22, wherein the first antibody or antibody fragment is a monoclonal antibody or antibody fragment, the second antibody or antibody fragment is a monoclonal antibody or antibody fragment, or the first and second antibodies or antibody fragments are monoclonal antibodies or antibody fragments.

109. The method according to claim 22, wherein the visually detecting includes conjugating a detectable label to the second antibody.

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~~110. The method according to claim 22, wherein the target cells are detected at a sensitivity of one target cell per 100 or more total cells.~~

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~~111. The method according to claim 22, wherein the second antibody is an IgG antibody and the first antibody recognizes the Fc-portion of the second antibody.~~

112. The method according to claim 48, wherein the first antibody or antibody fragment is a monoclonal antibody or antibody fragment, the second antibody or antibody fragment is a monoclonal antibody or antibody fragment, or the first and second antibodies or antibody fragments are monoclonal antibodies or antibody fragments.

113. The method according to claim 48, wherein the visually detecting includes conjugating a detectable label to the second antibody.

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114. The method according to claim 48, wherein the target cells are detected at a sensitivity of one target cell per 100 or more total cells.

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115. The method according to claim 48, wherein the second antibody is an IgG antibody and the first antibody recognizes the Fc-portion of the second antibody.

116. The method according to claim 87, wherein the target cells are detected at a sensitivity of one target cell per 100 or more total cells.

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